Fig.1

A Mouse ST8Sia VI

```
CGGAGCGGCGAGTCGGTGCCGCCCGGGCTGCGCTTCGCCCCGGCAGCTTTGGCGGCGAGGACGCCCGTGGCTCAGGATGAGATCGGGGGGCACGCTGTTC      100
                                                                  M  R  S  G  G  T  L  F                    8

GCCCTCATAGGCAGCCTGATGCTGCTGCTCCTCCTGCGTATGCTCTGGTGCCCAGCCGACGCGCCTGCCCGCTCCAGGCTGTTGATGGAGGGAAGCAGAG      200
A  L  I  G  S  L  M  L  L  L  L  L  R  M  L  W  C  P  A  D  A  P  A  R  S  R  L  L  M  E  G  S  R  E     42

AGGACACCAGTGGTACCTCAGCTGCACTGAAGACACTCTGGAGCCCGACAACCCCGGTACCACGCACCAGGAACAGCACATATCTGGATGAGAAGACAAC      300
  D  T  S  G  T  S  A  A  L  K  T  L  W  S  P  T  T  P  V  P  R  T  R  N  S  T  Y  L  D  E  K  T  T     75

CCAAATAACAGAGAAATGCAAAGATCTGCAATATAGCTTGAACTCTTTATCTAACAAAACGAGACGGTACTCTGAGGATGACTACCTCCAGACCATCACA      400
  Q  I  T  E  K  C  K  D  L  Q  Y  S  L  N  S  L  S  N  K  T  R  R  Y  S  E  D  D  Y  L  Q  T  I  T    108

AACATACAGAGATGCCCATGGAACCGGCAAGCAGAAGAATATGACAATTTTAGAGCAAAACTGGCTTCCTGTTGCGATGCCATTCAAGACTTCGTGGTTT      500
N  I  Q  R  C  P  W  N  R  Q  A  E  E  Y  D  N  F  R  A  K  L  A  S  C  C  D  A  I  Q  D  F  V  V  S    142

CCCAGAACAACACTCCAGTGGGGACTAACATGAGCTACGAGGTGGAAAGCAAGAAACACATCCCCATTCGAGAGAACATTTTCCACATGTTTCCAGTGTC      600
  Q  N  N  T  P  V  G  T  N  M  S  Y  E  V  E  S  K  K  H  I  P  I  R  E  N  I  F  H  M  F  P  V  S    175

GCAGCCTTTTGTGGACTATCCCTATAACCAGTGTGCAGTGGTTGGTAATGGGGGAATTCTCAACAAGTCTCTCTGCGGAGCAGAAATTGATAAATCTGAC      700
  Q  P  F  V  D  Y  P  Y  N  Q  C  A  V  V  G  N  G  G  I  L  N  K  S  L  C  G  A  E  I  D  K  S  D    208

TTCGTCTTCAGGTGTAACCTCCCCCCAATCACAGGGAGCGCTAGTAAAGATGTTGGAAGCAAAACAAATCTTGTGACTGTCAATCCCAGCATTATAACCC      800
F  V  F  R  C  N  L  P  P  I  T  G  S  A  S  K  D  V  G  S  K  T  N  L  V  T  V  N  P  S  I  I  T  L    242

TGAAGTACCAGAATTTGAAGGAGAAGAAAGCACAGTTTTTGGAGGACATCTCCACCTATGGAGATGCATTCCTCCTCCTGCCAGCATTTTCCTATCGGGC      900
  K  Y  Q  N  L  K  E  K  K  A  Q  F  L  E  D  I  S  T  Y  G  D  A  F  L  L  L  P  A  F  S  Y  R  A    275

CAACACAGGCATCTCTTTTAAAGTCTACCAAACACTCAAAGAGTCAAAAATGAGGCAAAAGGTTCTCTTCTTCCATCCCAGGTACCTGAGACACCTCGCT      1000
N  T  G  I  S  P  K  V  Y  Q  T  L  K  E  S  K  M  R  Q  K  V  L  F  F  H  P  R  Y  L  R  H  L  A     308

CTTTTCTGGAGAACTAAAGGGGTGACTGCATACCGCTTGTCCACAGGCTTGATGATTGCAAGTGTCGCTGTGGAACTGTGTGAAAACGTGAAGCTCTACG      1100
L  F  W  R  T  K  G  V  T  A  Y  R  L  S  T  G  L  M  I  A  S  V  A  V  E  L  C  E  N  V  K  L  Y  G    342

GATTCTGGCCTTTCTCTAAGACTATCGAAGACACCCCACTCAGTCACCACTACTATGATAACATGTTACCTAAGCATGGTTTCCACCAGATGCCTAAAGA      1200
  F  W  P  F  S  K  T  I  E  D  T  P  L  S  H  H  Y  Y  D  N  M  L  P  K  H  G  F  H  Q  M  P  K  E     375

ATACAGCCAAATGCTCCAGCTCCATATGAGAGGAATCCTCAAACTGCAATTCAGCAAATGTGAAACGGCTTAACGTTTCTTAGAAGGAGAATAATTTCAG      1300
Y  S  Q  M  L  Q  L  H  M  R  G  I  L  K  L  Q  F  S  K  C  E  T  A  *                                398

GAGGTGGAGTGGATGTGTCACAGCATCTCCAAAAAGCCAATAGAAGAAGGCACAGAGAAAGCATGAATTACAAAGGCGCTCTCCCACTTGTCTAGACCAA      1400
AGCCACCCGCCCCCACTCACTTTGCAGCCTCCACGAGTCACTCATTCTCACCTTCAACGTTCTTTCTCTGAGAATAGAGACCAAAACATCAGACTTGGAT      1500
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B Human ST8Sia VI

```
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                                                                  M  R  P                                  3

GGGGGCGCACTGCTCGCCCTGCTCGCCAGCCTGCTGCTGCTGCTGCTGCTGCGCCTGCTCTGGTGCCCGGCAGACGCGCCCGGCCGCGCCAGGATTCTGG      200
G  G  A  L  L  A  L  L  A  S  L  L  L  L  L  L  L  R  L  L  W  C  P  A  D  A  P  G  R  A  R  I  L  V     37

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  E  E  S  R  E  A  T  H  G  T  P  A  A  L  R  T  L  R  S  P  A  T  A  V  P  R  A  T  N  S  T  Y  L     70

GAATGAGAAGTCGCTCCAACTGACGGAGAAATGCAAAAATCTGCAATATGGCATTGAGTCTTTCTCTAACAAAACGAAAGGGTATTCAGAGAACGACTAC      400
N  E  K  S  L  Q  L  T  E  K  C  K  N  L  Q  Y  G  I  E  S  F  S  N  K  T  K  G  Y  S  E  N  D  Y     103

CTTCAGATTATCACAGATATACAGAGTTGTCCATGGAAACGGCAAGCAGAAGAATATGCAAATTTTAGAGCCAAACTTGCTTCCTGCTGTGATGCTGTTC      500
L  Q  I  I  T  D  I  Q  S  C  P  W  K  R  Q  A  E  E  Y  A  N  F  R  A  K  L  A  S  C  C  D  A  V  Q    137

AAAACTTTGTTGTTTCTCAGAATAACACTCCAGTTGGGACTAATATGAGTTACGAGGTGGAAAGCAAAAAAGAAATCCCAATTAAGAAGAACATTTTTCA      600
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TATGTTTCCAGTGTCCCAGCCTTTTGTGGACTACCCTTATAATCAGTGTGCAGTGGTCGGAAATGGGGGAATTCTGAATAAGTCTCTCTGTGGAACTGAA      700
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ATAGATAAATCCGACTTCGTTTTTAGGTGTAACCTACCCCCAACCACAGGAGATGTTAGTAAAGATGTTGGCAGTAAAACAAATCTTGTGACTATAAATC      800
I  D  K  S  D  F  V  F  R  C  N  L  P  P  T  T  G  D  V  S  K  D  V  G  S  K  T  N  L  V  T  I  N  P    237

CAAGCATCATAACTCTGAAATATGGGAACTTAAAGGAAAAAAAAAGCCCTATTTTTGGAGGACATTGCAACCTATGGAGATGCATTTTTTTTTCTGCCAGC      900
  S  I  I  T  L  K  Y  G  N  L  K  E  K  K  A  L  F  L  E  D  I  A  T  Y  G  D  A  F  F  F  L  P  A    270

ATTTTCCTTCAGGGCCAACACGGGTACCTCTTTCAAAGTATACTACACGCTCGAAGAGTCTAAAGCAAGACAAAAGGTTCTATTTTTCCATCCCAAGTAC      1000
F  S  F  R  A  N  T  G  T  S  F  K  V  Y  Y  T  L  E  E  S  K  A  R  Q  K  V  L  F  F  H  P  K  Y     303

CTGAAAGATCTGGCCCTTTTCTGGAGAACTAAAGGTGTGACTGCATACCGCTTGTCCACCGGCTTGATGATCACAAGTGTTGCAGTGGAACTGTGTAAAA      1100
L  K  D  L  A  L  F  W  R  T  K  G  V  T  A  Y  R  L  S  T  G  L  M  I  T  S  V  A  V  E  L  C  K  N    337

ATGTGAAGCTGTATGGATTCTGGCCCTTCTCTAAAACTGTAGAAGACATACCTGTCAGCCATCACTATTATGACAACAAGCTACCTAAACATGGTTTCCA      1200
  V  K  L  Y  G  F  W  P  F  S  K  T  V  E  D  I  P  V  S  H  H  Y  Y  D  N  K  L  P  K  H  G  F  H     370

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Q  M  P  K  E  Y  S  Q  I  L  Q  L  H  M  K  G  I  L  K  L  Q  F  S  K  C  E  V  A  *                398

AAAATGGGAATAATTTTAATATAATGCAGTAGGTGATTAACAATGTCTCCAAACACCAAAGGAGGTGGCTAAAGAGTATTTTGAGATGAGCCCCAAAATT      1400
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BEST AVAILABLE COPY

Fig.2

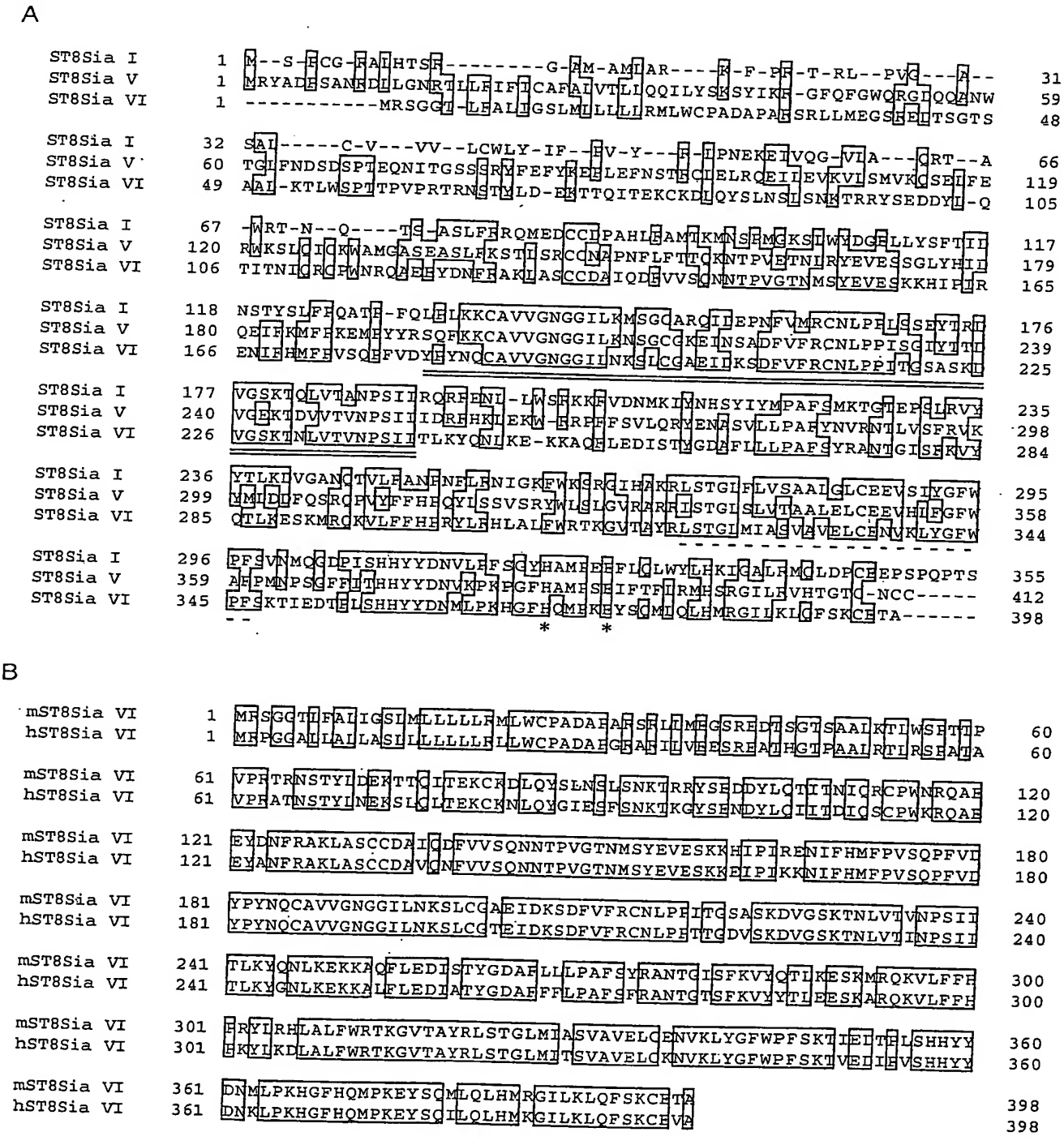


Fig.3

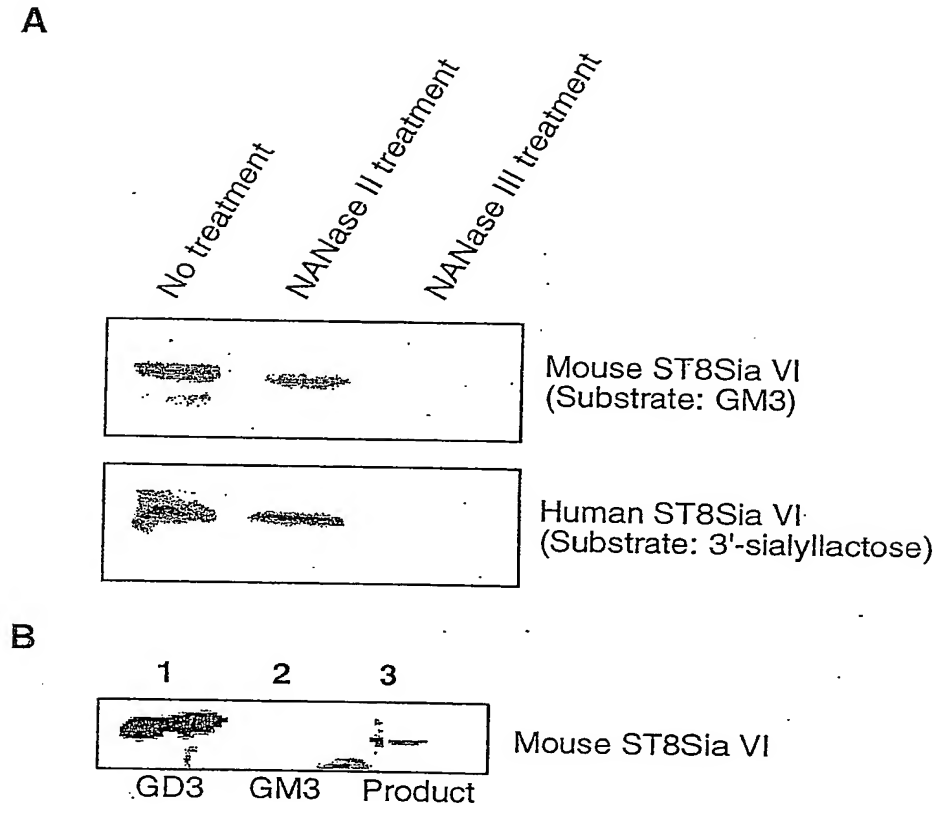
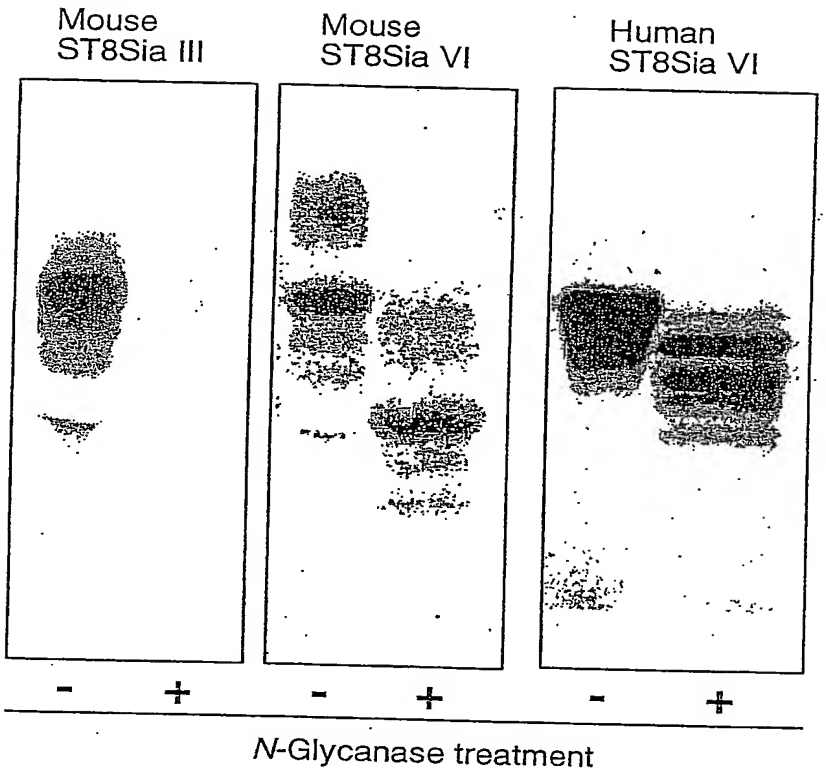

A
No treatment
NANase II treatment
NANase III treatment
Mouse ST8Sia VI
(Substrate: GM3)
Human ST8Sia VI
(Substrate: 3'-sialyllactose)
B
1
2
3
Mouse ST8Sia VI
GD3
GM3
Product

Fig.4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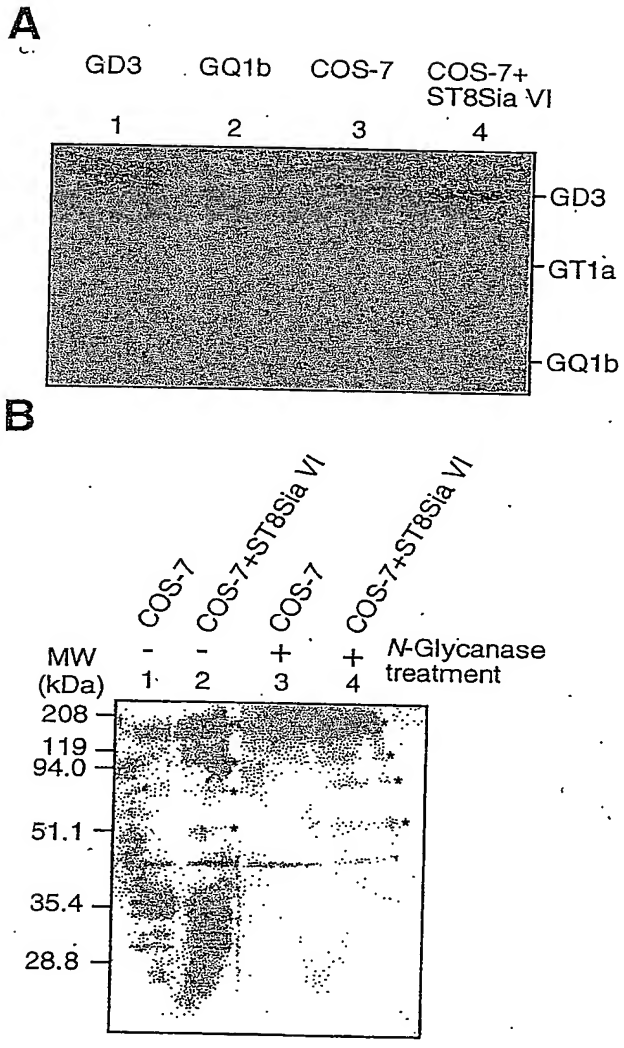
ST8Sia III
Mouse
ST8Sia VI
Human
ST8Sia VI
-
+
-
+
-
+
N-Glycanase treatment

Fig.5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GQ1b
COS-7
COS-7+
ST8Sia VI
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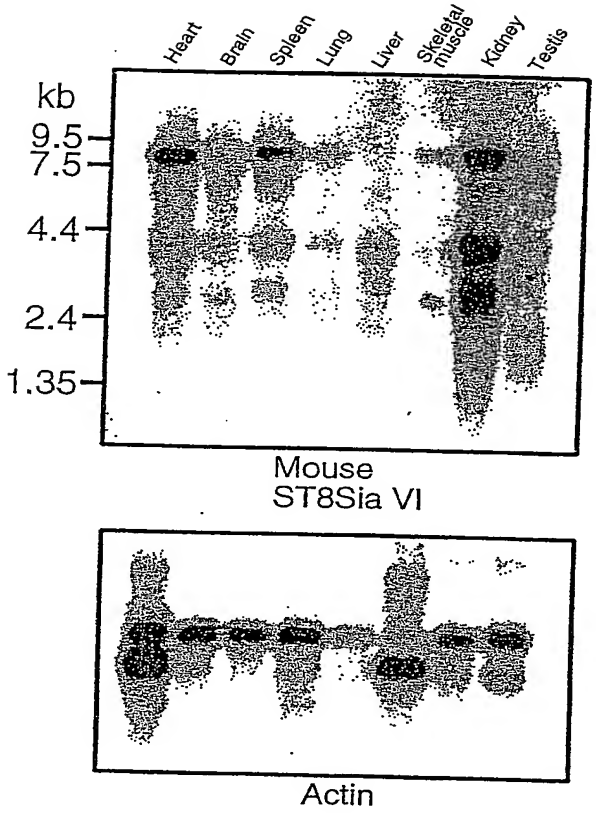
2
3
4
GD3
GT1a
GQ1b
B
COS-7
COS-7+ST8Sia VI
COS-7
COS-7+ST8Sia VI
MW
(kDa)
-
-
+
+
N-Glycanase
treatment
1
2
3
4
208
119
94.0
51.1
35.4
28.8

Fig.6

A


Heart
Brain
Spleen
Lung
Liver
Skeletal muscle
Kidney
Testis
kb
9.5
7.5
4.4
2.4
1.35
Mouse ST8Sia VI
Actin

B

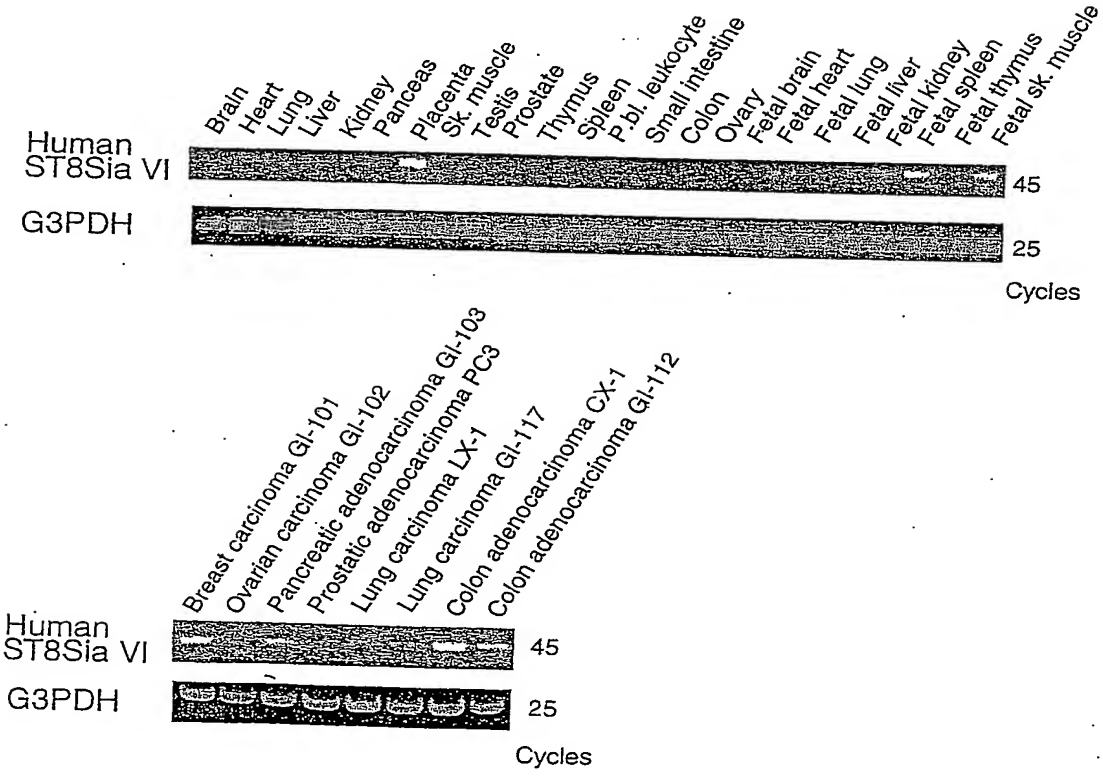

Brain
Heart
Lung
Liver
Kidney
Panceas
Placenta
Sk. muscle
Testis
Prostate
Thymus
Spleen
P.bl. leukocyte
Small intestine
Colon
Ovary
Fetal brain
Fetal heart
Fetal lung
Fetal liver
Fetal kidney
Fetal spleen
Fetal thymus
Fetal sk. muscle
Human ST8Sia VI
G3PDH
45
25
Cycles
Breast carcinoma GI-101
Ovarian carcinoma GI-102
Pancreatic adenocarcinoma GI-103
Prostatic adenocarcinoma PC3
Lung carcinoma LX-1
Lung carcinoma GI-117
Colon adenocarcinoma CX-1
Colon adenocarcinoma GI-112
Human ST8Sia VI
G3PDH
45
25
Cycles

Fig.7

A

Human ST6Gal II

```
ggcgccgggactccctcctggccgcccacagcctgtgcgcattcctgcattcctgccgccgcccgggacccgagcccccggaggtgtccaggcgcggtgc      100
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                                                                  M  K  P  H  L  K  Q  W  R                    9
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  L  S  F  L  E  T  R  R  L  L  P  V  Q  G  K  Q  R  A  I  M  G  A  A  H  E  P  S  P  P  G  G  L  D       75
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A  R  Q  A  L  P  R  A  H  P  A  G  S  F  H  A  G  P  G  D  L  Q  K  W  A  Q  S  Q  D  G  F  E  H  K      109
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  E  F  F  S  S  Q  V  G  R  K  S  Q  S  A  F  Y  P  E  D  D  D  Y  F  F  A  A  G  Q  P  G  W  H  S      142
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  E  A  G  L  S  R  A  Q  L  L  C  Q  L  R  S  R  A  R  V  R  T  L  D  G  T  E  A  P  F  S  A  L  G      275
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  L  G  E  E  I  D  S  H  D  A  V  L  R  F  N  S  A  P  T  R  G  Y  E  K  D  V  G  N  K  T  T  I  R      342
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I  I  N  S  Q  I  L  T  N  P  S  H  H  F  I  D  S  S  L  Y  K  D  V  I  L  V  A  W  D  P  A  P  Y      375
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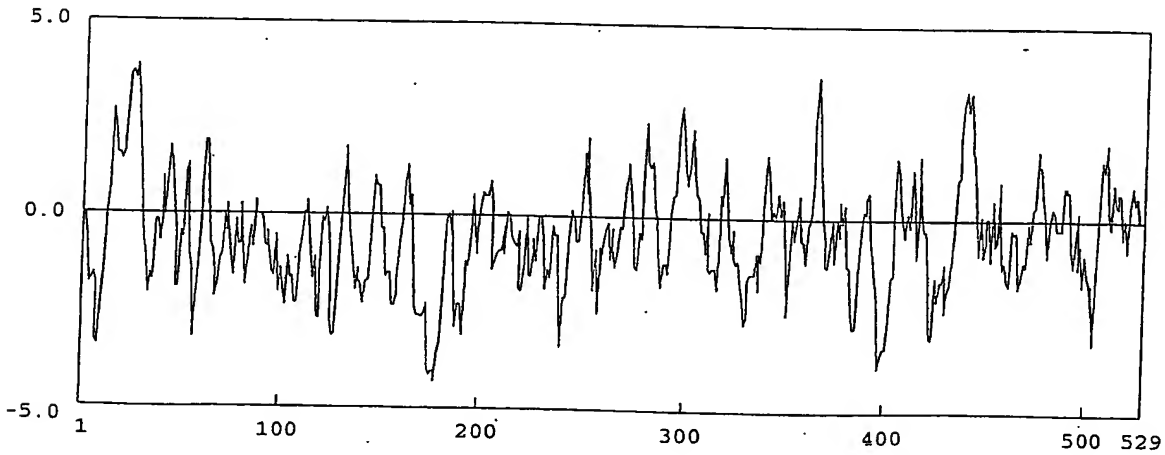

Fig.8

A

Mouse ST6Gal II

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  N  P  A  A  P  M  P  S  S  F  S  F  L  E  S  R  G  L  L  P  L  Q  G  K  Q  R  V  I  M  G  A  L  Q      66

GAACCCTCTTTGCCCAGAAGTTTGGATGCAAGCAAAGTGCTTCTGGACAGCCACCCTGAGAACCCTTTCCACCCTTGGCCTGGGGACCCACAGAAATGGG      300
  E  P  S  L  P  R  S  L  D  A  S  K  V  L  L  D  S  H  P  E  N  P  F  H  P  W  P  G  D  P  Q  K  W  D      100

ATCAGGCCCCAAATGGCTTTGACAATGGGGATGAGTTTTTTACATCCCAGGTTGGGAGGAAATCACAAAGCGCTTTCTATCCCGAGGAAGATAGCTATTT      400
  Q  A  P  N  G  F  D  N  G  D  E  F  F  T  S  Q  V  G  R  K  S  Q  S  A  F  Y  P  E  E  D  S  Y  F      133

TTTTGTTGCGGATCAGCCTGAGTTGTACCACCACAGGCAGGGTGCACTGGAGCTGCCATCTCCAGGGGAGACATCATGGCGATCAGGACCTGTTCAGCCC      500
  F  V  A  D  Q  P  E  L  Y  H  H  R  Q  G  A  L  E  L  P  S  P  G  E  T  S  W  R  S  G  P  V  Q  P      166

AAGCAGAAGCTGCTTCACCCAAGGCGAGGCAGCTTGCCTGAGGAAGCCTATGACAGCGACATGCTGTCAGCCTCCATGTCGAGAGCCTTCCTGTACCGGC      600
  K  Q  K  L  L  H  P  R  R  G  S  L  P  E  E  A  Y  D  S  D  M  L  S  A  S  M  S  R  A  F  L  Y  R  L      200

TCTGGAAGGGGGCCGTGTCCTCTAAGATGTTGAACCCGCGCCTGCAGAAGGCCATGCGTTACTACATGTCCTTCAACAAGCATGGTGTGCGCTTCCGCAG      700
  W  K  G  A  V  S  S  K  M  L  N  P  R  L  Q  K  A  M  R  Y  Y  M  S  F  N  K  H  G  V  R  F  R  R      233

GCGGGGTCGGCGTGAAGCTACACGTACAGGGCCGGAGCTGCTGTGTGAGATGCGCAGACGTGTGCGTGTGCGCACGTTGGACGGCAGAGAGGCGCCCTTC      800
  R  G  R  R  E  A  T  R  T  G  P  E  L  L  C  E  M  R  R  R  V  R  V  R  T  L  D  G  R  E  A  P  F      266

TCGGGGCTGGGCTGGCGGCCTCTGGTACCAGGTGTACCTCTGAGCCAGTTGCACCCGCGCGGTCTGAGCAGCTGCGCAGTTGTCATGTCTGCCGGTGCCA      900
  S  G  L  G  W  R  P  L  V  P  G  V  P  L  S  Q  L  H  P  R  G  L  S  S  C  A  V  V  M  S  A  G  A  I      300

TCCTGAACTCCTCCTTGGGGGAGGAAATCGATTCTCATGATGCAGTTTTGAGATTTAACTCTGCCCCTACCCGTGGCTACGAGAAAGATGTCGGAAATAA     1000
  L  N  S  S  L  G  E  E  I  D  S  H  D  A  V  L  R  F  N  S  A  P  T  R  G  Y  E  K  D  V  G  N  K      333

AACCACAGTACGCATCATTAATTCTCAGATTCTGGCCAACCCCAGCCATCACTTCATTGACAGTGCTTTATATAAAGATGTTATCCTGGTAGCCTGGGAT     1100
  T  T  V  R  I  I  N  S  Q  I  L  A  N  P  S  H  H  F  I  D  S  A  L  Y  K  D  V  I  L  V  A  W  D      366

CCTGCTCCTTATTCTGCCAATCTTAACCTGTGGTATAAGAAGCCAGATTACAACCTTTTCACTCCATATATCCAGCATCGCCGGAAATACCCGACTCAGC     1200
  P  A  P  Y  S  A  N  L  N  L  W  Y  K  K  P  D  Y  N  L  F  T  P  Y  I  Q  H  R  R  K  Y  P  T  Q  P      400

CATTTTACATTCTTCACCCCAAGTTCATATGGCAGCTTTGGGACATTATCCAGGAGAATACAAGGGAGAAGATACAGCCCAACCCACCATCTTCTGGTTT     1300
  F  Y  I  L  H  P  K  F  I  W  Q  L  W  D  I  I  Q  E  N  T  R  E  K  I  Q  P  N  P  P  S  S  G  F      433

TATTGGAATCCTCATCATGATGTCCATGTGTAAAGAGGTGCACGTGTATGAGTACATCCCATCTGTTCGACAGACAGAGCTTTGCCACTACCATGAGCTG     1400
  I  G  I  L  I  M  M  S  M  C  K  E  V  H  V  Y  E  Y  I  P  S  V  R  Q  T  E  L  C  H  Y  H  E  L      466

TACTACGACGCAGCCTGCACCTTGGGGGCCTACCACCCACTGCTCTATGAAAAGCTACTGGTGCAGCGCCTTAACACAGGCACCCAGGCAGACTTGCATC     1500
  Y  Y  D  A  A  C  T  L  G  A  Y  H  P  L  L  Y  E  K  L  L  V  Q  R  L  N  T  G  T  Q  A  D  L  H  H      500

ACAAGGGCAAGGTAGTCTTGCCAGGCTTCCAGACCCTTCGGTGTCCAGTAACCAGCCCCAACAATACACATTCTTAAAATGGAACTCTTGGGAACTGATG     1600
  K  G  K  V  V  L  P  G  F  Q  T  L  R  C  P  V  T  S  P  N  N  T  H  S  *                              524

TGCAATAAGGT                                                                                              1611
```

B

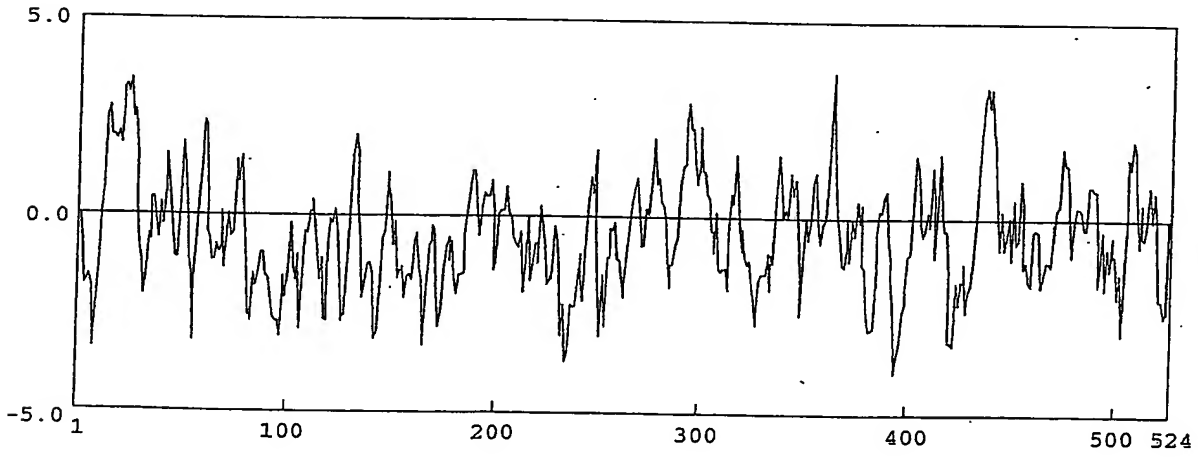


Fig.9

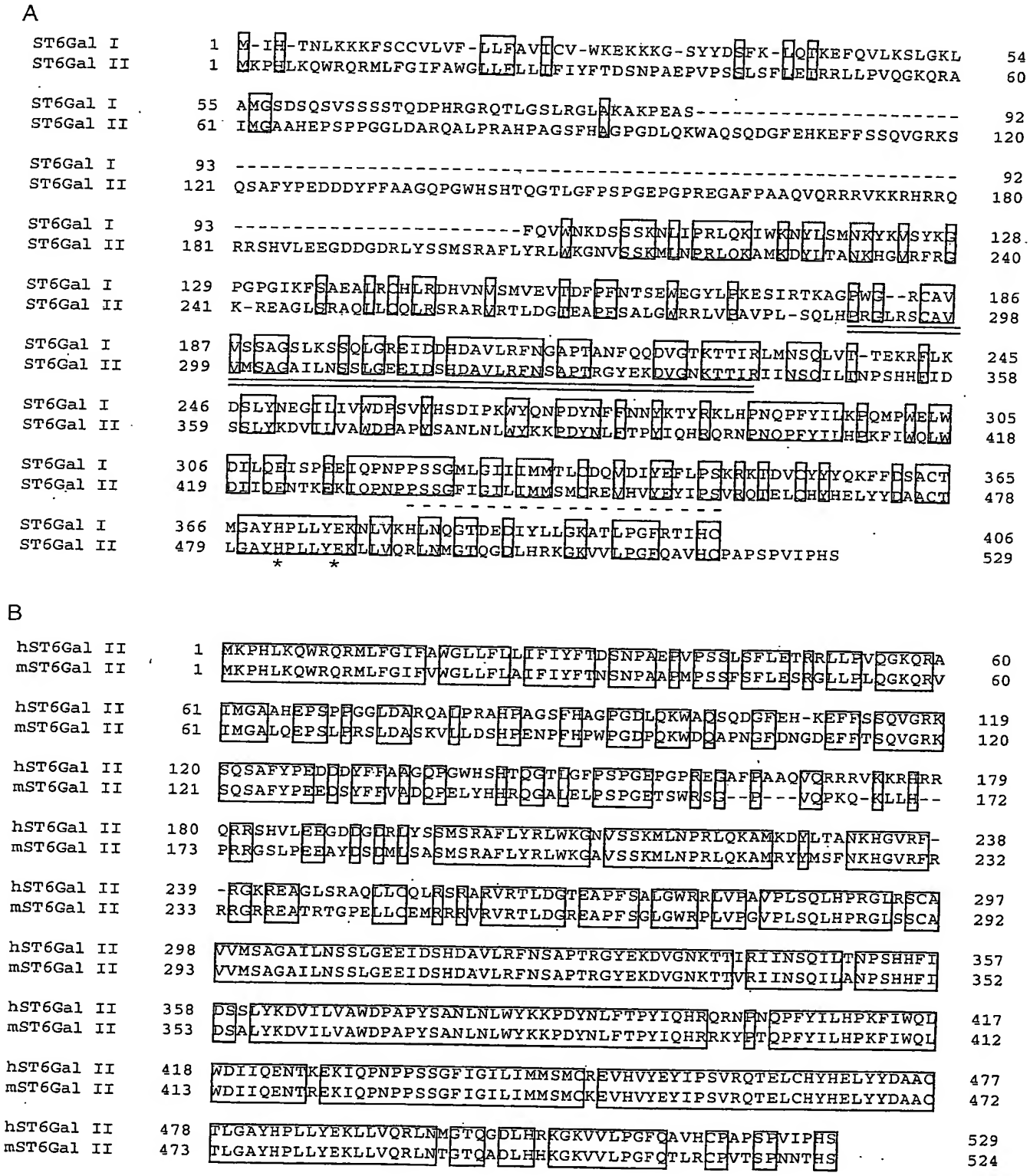


Fig.10

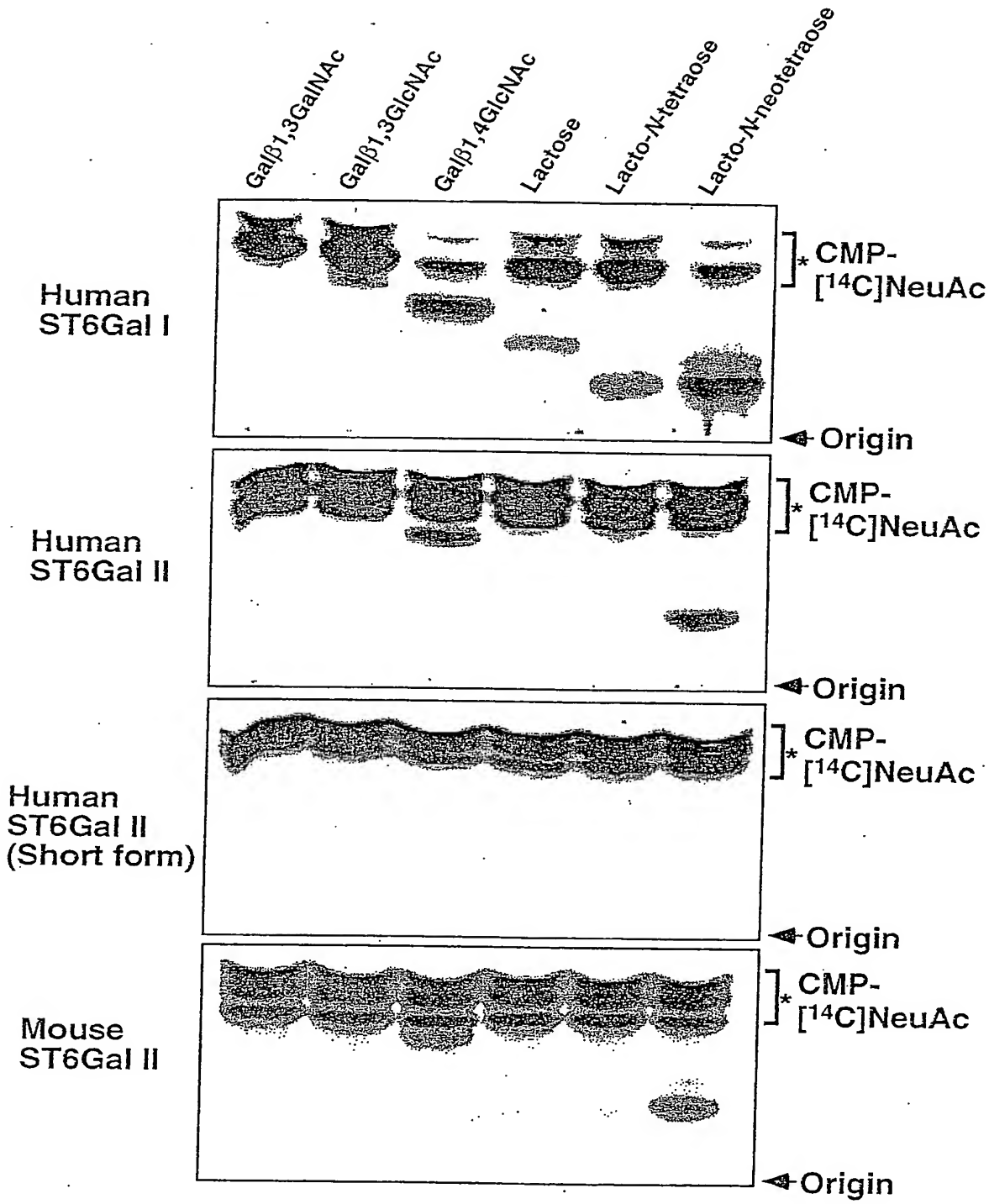

Galβ1,3GalNAc
Galβ1,3GlcNAc
Galβ1,4GlcNAc
Lactose
Lacto-N-tetraose
Lacto-N-neotetraose
Human ST6Gal I
Human ST6Gal II
Human ST6Gal II (Short form)
Mouse ST6Gal II
*CMP-[14C]NeuAc
Origin

Fig.11

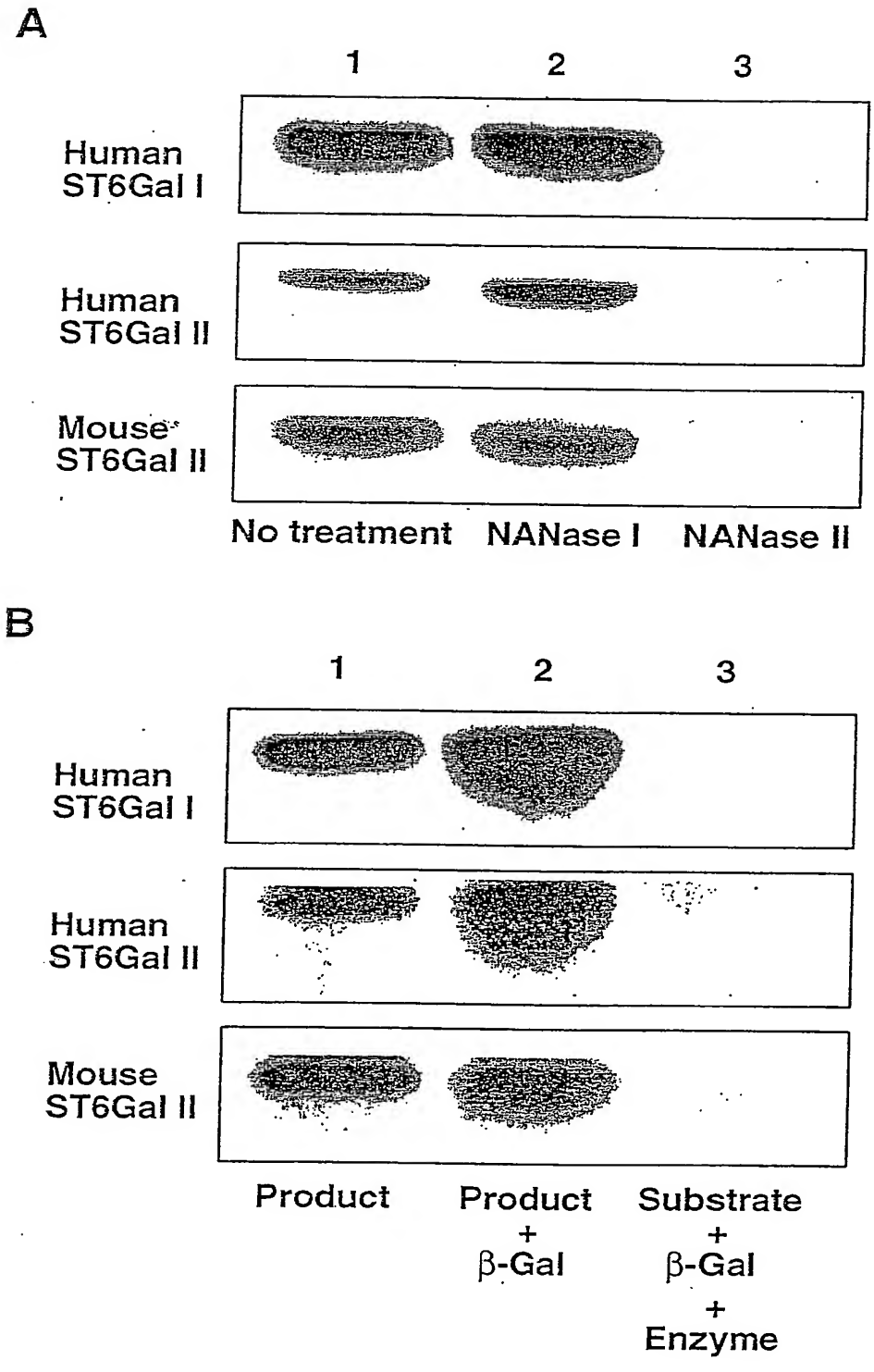
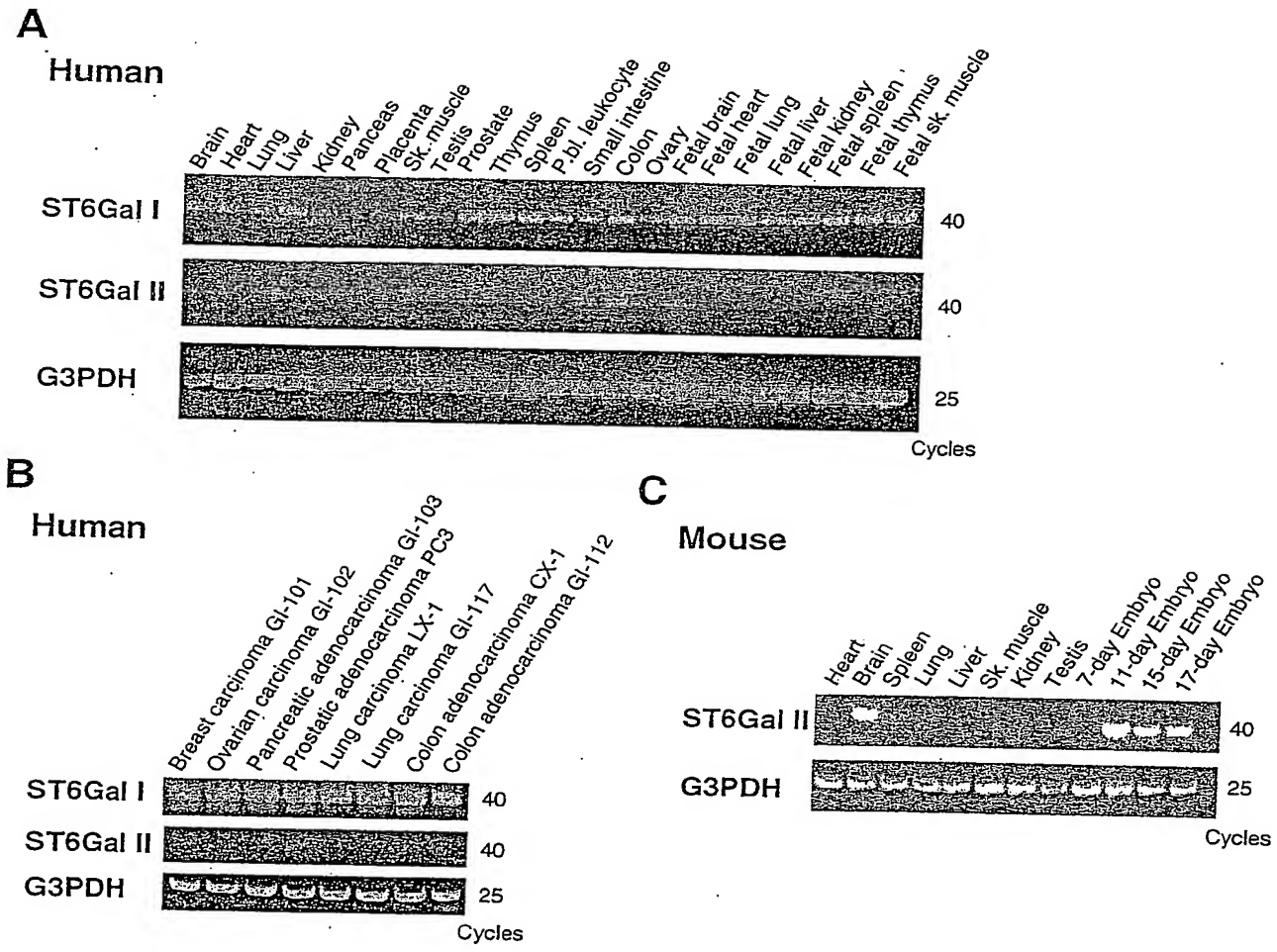

A
1
2
3
Human ST6Gal I
Human ST6Gal II
Mouse ST6Gal II
No treatment
NANase I
NANase II
B
1
2
3
Human ST6Gal I
Human ST6Gal II
Mouse ST6Gal II
Product
Product + β-Gal
Substrate + β-Gal + Enzyme

Fig.12


A
Human
Brain
Heart
Lung
Liver
Kidney
Panceas
Placenta
Sk. muscle
Testis
Prostate
Thymus
Spleen
P.bl. leukocyte
Small intestine
Colon
Ovary
Fetal brain
Fetal heart
Fetal lung
Fetal liver
Fetal kidney
Fetal spleen
Fetal thymus
Fetal sk. muscle
ST6Gal I
ST6Gal II
G3PDH
40
40
25
Cycles
B
Human
Breast carcinoma GI-101
Ovarian carcinoma GI-102
Pancreatic adenocarcinoma GI-103
Prostatic adenocarcinoma PC3
Lung carcinoma LX-1
Lung carcinoma GI-117
Colon adenocarcinoma CX-1
Colon adenocarcinoma GI-112
ST6Gal I
ST6Gal II
G3PDH
40
40
25
Cycles
C
Mouse
Heart
Brain
Spleen
Lung
Liver
Sk. muscle
Kidney
Testis
7-day Embryo
11-day Embryo
15-day Embryo
17-day Embryo
ST6Gal II
G3PDH
40
25
Cycles

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